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Targeted cytotoxic somatostatin analog AN-162 inhibits growth of human colon carcinomas and increases sensitivity of doxorubicin resistant murine leukemia cells

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ABSTRACT

The effect of the targeted cytotoxic somatostatin (SST) analog AN-162, consisting of doxorubicin (DOX) conjugated to SST carrier RC-121, was investigated on the growth of human colorectal cancer (CRC) cell lines HT-29, HCT-15, and HCT-116 and a DOX-resistant mouse leukemia cell line P388/R84. mRNA for SST-receptors and high affinity binding sites for SST were detected in all CRC cell lines and in P388/R84 cells. In contrast to DOX alone, AN-162 blocked HCT-116 cells and P388/R84 cells in S/G2 phase and increased the number of apoptotic cells. In vivo, AN-162 reduced the volume of CRC xenografts more effectively than its unconjugated components. Our results suggest that AN-162 inhibits growth of experimental CRC more effectively than DOX and increases sensitivity of DOX resistant human leukemia cells.

1. Introduction

Colorectal cancer (CRC) is the third commonest cause of cancer-related deaths among men and women in the Western world [1,2]. At the time of presentation, about ~40% of patients already have distant metastases. Chemotherapeutic regimens based on 5-fluorouracil (5-FU) are commonly used for treatment of patients with advanced CRC. Although the combinations of 5-FU with Irinotecan and Oxaliplatin have improved the response rate, chemotherapeutic regimens are usually not very effective against disseminated CRC due to multiple drug resistance (MDR). Thus, the development of new therapeutic agents that can overcome this resistance has become one of the most important goals in the management of this malignancy. The elucidation of specific molecular characteristics of tumor cells led to the development of a new treatment strategy known as targeted therapy. Modern targeted

Abbreviations: SST, somatostatin; DOX, doxorubicin; CRC, colorectal cancer; MDR, multiple drug resistance; MTS, 3-(45-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium; TopII, Topoisomerase II; Pgp, P-glycoprotein; MRP-1, MDR related protein-1.

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anticancer drugs include antibodies against surface structures on malignant cells and conjugates consisting of receptor-specific ligands linked to toxins, radionuclides, or chemotherapeutic agents [3,4]. Because antineoplastic drugs can be delivered directly to cancer cells, their higher intra tumoral concentration is expected to result in greater antitumor efficacy with reduced systemic toxicity and may overcome the chemoresistance of some malignant cells. In our endeavour to develop chemotherapy targeted to receptors, we synthesized cytotoxic hybrids of somatostatin (SST) containing DOX or the highly active derivative of DOX; 2-pyrrolino-DOX, conjugated to the octapeptide SST analogs RC-160 or RC-121 [5]. These SST analogs, which were also developed in our institute [6], exert their effects through G-protein coupled receptors [7]. At least five distinct SST receptor subtypes SSTR1-5 have been characterized [7]. RC-160 and RC-121 display high affinity to SSTR2 and 5, moderate affinity to SSTR3, and only poor binding to SSTR1 and SSTR4 [7–9]. In a previous study with human colon carcinomas, we showed a strong inhibition of tumor growth after treatment with the targeted cytotoxic somatostatin analog AN-238, containing 2-pyrrolino-DOX linked to RC-121 [10]. Recently, another cytotoxic SST analog AN-162 [AEZS-124] consisting of DOX linked to SST octapeptide RC-121 has shown significant antineoplastic activity and favourable toxicity profile in lung and breast cancer [11]. Consequently, we examined AN-162 in vitro and in vivo in SSTR-positive HT-29, HCT-15 and HCT-116 human colon cancer cell lines. In addition, we investigated whether targeting could impair chemoresistance in a DOX-resistant mouse leukemia cell line P388/R84 in vitro.

2. Material and methods

2.1. Peptide and chemicals

Cytotoxic somatostatin analog AN-162 [AEZS-124], first synthesized in our laboratory, was provided for this study by Aeterna Zentaris GmbH, Frankfurt am Main, Germany [5]. For in vitro and in vivo use AN-162 was dissolved in 0.01 N acetic acid and diluted with 5% Mannitol. SST analog, RC-160 (Vapreotide acetate), first synthesized by our laboratory was provided by Genzyme (Cambridge, MA). RC-160 is equivalent to RC-121 and was used instead of RC-121, which was not available for this study [12,13]. Doxorubicin hydrochloride (DOX) was obtained from Chemtec Leuna GmbH (Leuna, Germany).

2.2. Cell culture

The human colon cancer cell lines HT-29, HCT-15 and HCT-116 were obtained from the American Type Culture Collection (ATCC). The DOX-resistant mouse leukemia cell line P388/R84 was developed by one of us (AK) and was described previously [14]. The P388/R84 cell line was established by growth of P388 mouse leukemia cells in the continuous presence of 2 μg/ml of DOX for 2 weeks in soft agar. Colonies surviving this treatment were collected and grown in suspension cultures without DOX. In soft agar assays, the P388/R84 cell line was 84-fold more resistant to DOX than the parental P388 cell line.

Cultures were grown in growth media, McCoys (HT-29 and HCT-116) or RPMI-1640 (HCT-15 and P388/R84), containing 10% fetal calf serum (FCS) and maintained at 37 °C in a humidified atmosphere containing 5% CO_2. The media were supplemented with 100 IU/ml of penicillin and 100 μg/ml of streptomycin. Media for P388/R84 was additionally supplemented with 10 μM 2-mercaptoethanol.

2.3. RNA isolation and reverse-transcriptase (RT-) PCR

Total RNA was isolated and DNAse treated using the Macherey–Nagel NucleoSpin kit according to the manufacturer's instructions (Macherey–Nagel, Germany) then reverse transcribed into cDNA by Moloney murine leukemia virus reverse-transcriptase using random primers (Promega) in a final volume of 20 ml. A negative control, with no reverse-transcriptase added, was also included. RT-PCR was performed as previously described [11], except for SSTR4, where 0.20 μmol/l of each primer were used and the annealing temperature was 57 °C. The integrity of cDNA was tested by RT-PCR for glyceraldehyde-3-phosphate dehydrogenase (GAPDH). All PCR reactions were done in an Applied Biosystems 2700 thermal cycler (Applied Biosystems, Foster City, CA). Ten-microliter of each amplification reaction was electrophoretically separated on a 1.5% agarose gel, stained with ethidium bromide, and visualized under UV light. Gene-specific primers for SSTR1, SSTR2, SSTR3, SSTR4, SSTR5 and GAPDH were as described previously [11].

2.4. Receptor binding studies

The binding characteristics of receptors for SST were determined in membrane fractions of HT-29, HCT-15, HCT-116 and P388/R84 cells as described previously [11]. For in vitro ligand competition assays, radioiodination of SST analog RC-160 and separation of the mono-iodinated radioligand by HPLC were carried out.

2.5. Cell viability assay (MTS assay)

Cell viability was determined by using the 3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium;(MTS) assay kit (CellTiter 96 Aqueous One Solution Cell Proliferation Assay, Promega Corp., Madison, WI, USA) according to the manufacturer's instructions. Briefly, cells were seeded into 96-multiwell plates (Becton Dickinson and Co., Franklin Lakes, NJ, USA) at a density of 5×10^3 cells per well in 100 μl of culture medium. After 24 h of incubation, the medium was replaced with fresh medium containing indicated concentrations of AN-162 or DOX and incubated for 72 h. Finally 20 μl of MTS solution was added to each well and incubated for an additional 2 h. Mitochondrial dehydrogenase enzymes of viable cells converted MTS tetrazolium into a colored formazan product. The optical density of samples was measured at 550 nm using a Dynax Plate reader. Experiments were performed in hexaplicates and repeated three times.